FINAL REPORT

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DEVELOPMENT OF ZERO ERUCIC ORIENTAL MUSTARD

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Title: "Development of Zero Erucic Acid, Oriental Mustard with Improved Agronomic Adaptation and Seed Quality Traits"

Summary

The first objective is to develop 'zero' erucic acid oriental mustard (ZEM) with agronomic and seed quality traits that are competitive with that of standard oriental mustard varieties. The second objective is to initiate development of ZEM with a canola-type fatty acid profile that will improve ZEM oil's value and marketability as an edible oil product.

A large seeded ZEM line was crossed with cultivar AC Vulcan and backcrossed to AC Vulcan. Selection in F_2 and BC_1F_2 generation produced zero erucic acid lines. These lines and progeny have been evaluated in three successive field nurseries (1998, 1999, and 2000) and two successive replicated yield trials (1999, 2000). Selection criteria for later trials were agronomic evaluation (observations during the growing season, maturity and plot yield) and seed quality parameters (decreased oil, increased protein, increased seed 1000 seed weight, increased allyl glucosinolate, improved seed colour, etc.). Lines meeting these criteria were advanced into replicated yield trials and new nurseries created using plants harvested one replicate of the selected lines of the previous year's nurseries. In 2000 as well two ZEM lines were entered into the 2000 co-operative mustard trial.

Also interspecific lines of *Brassica juncea* having a canola-type fatty acid profile and/or carrying resistance to white rust race 2v (resulting from an interspecific cross with low linolenic acid, *Brassica napus*) were crossed to ZEM or AC Vulcan. Subsequent generations are being selected for canola-type fatty acid profile, for resistance to white rust race 2v and for a normal oriental mustard glucosinolate content and are being further backcrossed to ZEM. In 2000 selection for improved fatty acid profile continued in the greenhouse and lines having an altered fatty acid content resulting from this interspecific cross were evaluated in a field nursery. And in 2000 two lines of white resistant resistant (race 2v) oriental mustard was entered into the 2000 co-operative mustard trial.

Technical Report

A. Background and objectives

Mustard production has increased in western Canada over the last 30 years. Production for the last five years has averaged 253,000 tonnes with a seeded area of 272,000 hectares. Presently, most oriental mustard acreage in western Canada is in Saskatchewan (80%). Mustard is comprised of two species: *Brassica juncea* (Brown and Oriental mustard) and *Sinapis alba* (Yellow mustard). Condiment mustard is used as dry mustard or prepared mustard. Yellow Mustard flour is also used as an ingredient in prepared meats. In Saskatchewan oriental mustard is the second most important mustard grown behind Yellow mustard. Oriental mustard is the highest yielding of the three mustards. Canadian condiment mustard cultivars of *B. juncea* are well adapted to dryland agriculture on the southern prairies. They are higher yielding than canola cultivars, resistant to blackleg, early maturing, drought tolerant and resistant to seed shattering. However, present day varieties are only suitable for the condiment trade as their seed oil contains high amounts of erucic acid. Some mustard seed processors, particularly those mustard processors in Japan (a major purchaser of Canadian oriental mustard) remove approximately one-half of the oil when preparing their condiment product. The by-product (oil) is of little value because it is neither an edible oil (erucic acid content too high, 20-25%) nor good a source of erucic acid for the industrial market (>50% erucic acid required). Zero erucic acid containing lines of oriental mustard (ZEM) do exist, however their agronomic performance is poor. As the mustard seed processors are unwilling to pay a high premium for this seed, without an improvement in the agronomic performance of these lines of zero erucic acid oriental mustard are not an alternative for the Saskatchewan farmer as yet.

Even if the oil of zero erucic acid mustard were available in the marketplace it would not be considered quality edible oil for all applications. This is because ZEM oil is quite unsaturated, which is associated with a decreased

stability of the oil and a short shelf life. It may be considered useful as a salad oil, but as cooking oil it would soon develop a fishy odour and turn rancid because of high level of linolenic acid present. For margarine it would be too unsaturated and would require a higher degree of hydrogenation than canola. In order for zero erucic acid mustard oil to be considered top quality edible oil its fatty acid profile needs to be altered so that it is similar to canola oil.

This project has two objectives. The first objective is to develop a zero erucic cultivar of oriental mustard with a yield potential similar to that standard oriental mustard varieties registered at the present time. This represents a requirement for a 5-10% increase in yield. In addition to yield, selections for a lower fixed oil content, lower chlorophyll content, higher protein content and higher allyl glucosinolate content (all desirable seed quality traits to the mustard industry) will be made. This will be accomplished via a cross of a zero erucic mustard line with AC Vulcan, a high yielding cultivar of oriental mustard in commercial production today. Followed by reselection of the zero erucic acid trait and selection under field conditions for lines with improved yield and other agronomic indicators, and improved seed quality traits.

The second objective is to initiate the development of an oriental mustard line with a fatty profile similar to canola. The goal is to develop a line that has a relatively high content of oleic acid (>55%, compared to 40-48% for ZEM) and a relatively low content of linoleic acid (<25%, compared to 29-36% for ZEM) and linolenic acid (<10%, compared to 13-17% for ZEM) in its oil. This objective will be accomplished by taking advantage of the fact that interspecific crosses of *Brassica juncea* with low linolenic acid *Brassica napus* have already been made at the AAFC-Saskatoon Research Centre in order to develop canola quality *Brassica juncea* into a third species of canola. From these interspecific crosses *Brassica juncea* lines have been selected that contain a high content of oleic acid and a low content linolenic acid. Unfortunately these lines of low linolenic *B. juncea* are very weak and contain a low concentration of glucosinolates, particularly allyl glucosinolate which is the glucosinolate found in oriental mustard and is a essential flavour of derived mustard. Thus these lines can not be considered oriental mustard. By crossing and backcrossing these lines with oriental mustard followed by reselection of the high oleic acid / low linolenic acid trait it is expected that a line of zero erucic, high oleic, low linolenic acid oriental mustard can be developed. By the end of this project these lines will not necessarily be equal to standard oriental mustard in performance, but should rather be viewed as the development of parental material from which new improved cultivars of ZEM can be developed. It is expected that they will survive to maturity under field conditions however. Also by taking advantage of the natural immunity in *Brassica napus* to *Albugo candida* (white rust), it is expected that resistance to the recently discovered race 2v can be introgressed into oriental mustard via crosses of the interspecific *B. juncea* by *B. napus* to oriental mustard. Previously, resistance to white rust race 2v was not known occur in oriental mustard. This development will eventually lead to ZEM cultivars with an increased ability to fight disease and increased stability of production for the Canadian farmer.

B. Project activities, achievements, papers written for 2000

Objective 1 - Development of a ZEM cultivar

This part of project was initiated by crossing a zero erucic acid, large seeded, oriental mustard line J93-0877 with AC Vulcan. J93-0877's seed size is almost double that of registered oriental mustard cultivars (averaged 4.1 grams / 1000 seed compared to 2.5 grams / 1000 seed in the 1997 Co-operative Mustard Trials) and it has a low fixed oil content, both positive traits for an oriental mustard. But it is relatively low yielding, is late maturing, has a tendency to produce seed with a high chlorophyll content (high green seed count), has a low protein content, and it has a relatively low allyl glucosinolate content (low volatile oil content) compared to standard oriental cultivars. AC Vulcan is one of the highest yielding, has the highest allyl glucosinolate content of all oriental mustard cultivars available today and highest protein content. It is also early maturing (chlorophyll is not usually a problem with this cultivar) and carries resistance to the disease *Albugo candida* (white rust) race 2a. It is susceptible to white rust race 2V, as are all varieties of oriental mustard and its' oil content tends to be higher than ideal. By combining the best traits of both lines it may be possible to develop a zero erucic, large seeded, low oil, high protein, high allyl glucosinolate oriental mustard with the field performance approaching that of AC Vulcan.

Previously, a cross between J93-0877 and AC Vulcan was made and the F_1 backcrossed to AC Vulcan. Selection for zero erucic acid content and large seed size was done in the F_2 and BC_1F_2 generations. In 1998 F_3 and BC_1F_3 seed from 186 selected zero erucic plants was used to plant a three replicate single row field nursery. After agronomic and seed quality evaluations were completed selected lines were entered into a three location replicated yield trial in 1999. Also individual plants were harvested from these selected rows were used to create a field nursery in 1999 of 302 BC_1F_4 and F_4 lines to initiate a second cycle of selection for better performance, decreased oil, etc. Following seed quality evaluation in spring 2000, one ZEM line (J98-326) was selected for entry into the 2000 co-operative

mustard trial and 21 lines were selected from the 1999 field nursery for entry into a multilocation trial in 2000. A third nursery of 198 selected lines was also created in 2000 from 10 individual plants harvested from 20 selected entries of the 1999 nursery. A final multilocation trial of 18 selected lines from the 2000 field nursery has been created for 2001. One line from the 2000 multilocation trial will be entered into the 2001 co-operative mustard trial for further evaluation and possible registration as a ZEM cultivar.

An another cross was made between a ZEM line and Common Brown mustard. The focus of this cross was development of an improved cultivar of brown mustard. However, the yellow seeded lines segregating from this cross were kept and evaluated against AC Vulcan in a field nursery. The best performing lines were advanced into a multilocation replicated yield trial and in 2000 one line (J97-102) was entered into the co-operative mustard trial. Summary data for this line is also presented in the results and conclusions section.

Objective 2 - Development of high oleic / low linolenic acid ZEM

Initially, early generation lines of high oleic/linolenic acid (canola-type fatty acid profile), low glucosinolate *B. juncea* derived from an interspecific cross with *B. napus* was crossed with AC Vulcan and backcrossed to AC Vulcan. Selection for oriental mustard with a canola-type oil in the successive generations was unsuccessful, but selection for resistance to white rust 2V was. Therefore in 1998 a three-replicate, single row, field nursery of 105 lines was grown for agronomic evaluation. Generally the performance of the entries was good compared to AC Vulcan and Forge. Several lines rated better than or equal to Forge in agronomic performance and several lines had a low oil content similar to Forge. Selected lines from this nursery were entered into a multilocation yield trial in 1999 to be further evaluation and two lines were entered into 2000 co-operative mustard trial. The top ranked line of this trial will be crossed to ZEM to develop zero erucic acid lines resistant to race 2V of white rust.

A second attempt at introgressing the high oleic, low linolenic acid fatty acid profile of *B. napus* into ZEM was initiated. A cross of zero erucic acid BC_1F_2 lines [(J93-0877 x AC Vulcan) x AC Vulcan of objective 1] with later generation lines (BC_4F_4) of high oleic/linolenic acid, low glucosinolate *B. juncea* derived from an interspecific cross with *B. napus* was made. This material was advanced through successive generation in the greenhouse by half-seed selection for a fatty acid composition of >55% oleic acid and <8% linoleic acid, followed by selection for high allyl glucosinolate content using leaf analysis. Confirmation of fatty acid profile and glucosinolate content was done by analysis 10 of the seeds harvested selected plants. A progeny evaluation of 76 F_3 lines was completed in a 1999 field nursery. Progeny evaluation of 54 F_4 lines was completed in 2000. Harvested seed of these nurseries was used for evaluation of the progress of this project. Individual plants were also harvested from entries of the 1999 and 2000 nurseries and some of these, following fatty acid analysis were used for either further advancement in the greenhouse or for further progeny evaluation. Meanwhile in 2000 in the greenhouse, F_5 seed were produced from selected F_4 lines by half-selection and leaf analysis. Unfortunately, insufficient seed for a field trial was produced. The selected F_4 lines were crossed to a large seeded, ZEM selection from objective 1 of this project to improve the agronomic performance of this material. BC_1F_1 seed has been produced.

Another cross of zero erucic acid BC_1F_2 lines [(J93-0877 x AC Vulcan) x AC Vulcan of objective 1] with early generation lines (BC_1F_1) of high oleic/linolenic acid, low glucosinolate *B. juncea* derived from an interspecific cross with *B. napus* were also initiated in 1998. These crosses were followed through successive backcross generations using the molecular markers for linolenic acid content Dr. Somers developed from a *B. napus* mapping population. This is being done to see if this technology can be extrapolated to *B. juncea* for the selection of high oleic/low linolenic acid ZEM. If this approach is successful, the lines of low linolenic ZEM developed may also represent a further enhancement over the lines developed as described in the previous paragraph in terms of seed quality. This material was advanced to the BC_4F_1 generation in the greenhouse. The BC_4F_2 seed was planted in a nursery in 2000 together with checks. Individual plants in this nursery were bag selfed; tissue sampled for DNA marker analysis and individually harvested. The fatty acid composition of the seed oil of these plants was determined and compared with the presence or absence of the low linolenic acid B. napus markers to determine the effectiveness of this approach.

No paper on this aspect of the project was written in 2000. One poster paper on white rust resistant (race 2v) *Brassica juncea* was presented at the 10th International Rapeseed Congress held in Canberra, Australia, September 26-29, 1999.

Title: Resistance to white rust in *Brassica Juncea* from an interspecific cross with *B. napus*.
Authors: Richard K Gugel, J Philip Raney, Lorne J Duczek, Gerhard FW Rakow and Todd V Olson

A copy of this poster was included with a previous report.

C. Results and conclusions for 2000

Goal 1 - Development of a ZEM cultivar

This aspect of the project has generally been progressing well. Analysis of the harvested seed from the 1999 field nursery for various seed components was completed. From the results obtained from this nursery, selections for entry into a multilocation yield trial and a second field nursery were made. Selection for entry into these tests was on the basis of a straight stem morphology (top priority), maturity, good agronomic rating, low oil and high protein content.

The unusual twisted stem morphology first observed by Dr. Rakow in many lines in the 1999 nursery and replicated trial was also observed in several lines in 2000, but it appeared that selection against this trait in 1999 nursery was largely successful. Of the 20 F_3 families evaluated in the 2000 field nursery 11 families were essentially free of this trait. Another 7 had 3 or less of the 10 F_4 entries within the family displaying this trait. 13 of 21entries in the 2000 replicated trial were also okay. The J93-0877 parent check again displayed severe problems with this morphology whereas the AC Vulcan parent was relatively clean. It is therefore speculated that this twisted stem morphology has a genetic basis. Therefore the only lines that will be advanced from the 2000 trials will be lines free of this morphology. Otherwise both the nursery and the replicated trial were good trials and lines were found with performances comparable to AC Vulcan.

Table 1 is a listing of the data generated from the 2000 multilocation replicated trial. The maturity of these entries is generally within 1 to 2 days of AC Vulcan (4 to 6 days earlier than TO03-0877). The oil contents of these lines are generally lower than the checks (up to 4% lower than AC Vulcan. The one thousand seed weights of all entries are larger than AC Vulcan and Forge. Entry 16 has been entered into the 2001 co-operative mustard trial.

Table 2 contains a summary of the performance of ZEM entries in the 2000 co-operative mustard trial. The performance of the entries was quite good. However, because of the low glucosinolate content and unacceptable chlorophyll content, J98-326 has been dropped from the co-operative mustard. J97-102 remains in the trial.

Table 1 Data Collected on entries in 2000 multilocation replicated yield trial.[a]

Entry	DTM (days)	Height (cm)	Yield (kg/ha)	Oil (% Dry)	Protein (% Dry)	Seed Wt (gm/1000)	Field Notes	Colour (WIE)	Allyl G.[b] (µmole/g)
1	96	143	2820	43.7	27.7	3.26	Twist	-36.5	75
2	96	139	2790	40.2	27.9	3.04		-39.6	92
3	96	154	2703	39.3	28.2	3.24	Staghead	-42.2	118
4	96	146	2885	38.4	29.2	3.20		-41.3	106
5	95	150	2745	38.0	28.7	3.29	Staghead	-39.7	104
6	96	149	2485	36.9	29.2	3.78	Twist	-37.8	107
7	96	146	2755	38.4	29.0	3.23	Twist	-41.9	107
8	96	148	2782	39.0	28.1	3.15	Staghead	-40.6	102
9	96	149	2731	37.7	29.1	3.29		-40.5	111
10	96	152	2650	36.6	29.6	3.60	Twist	-40.3	115
11	95	148	2676	38.5	29.1	3.15		-41.1	113
12	97	147	2638	39.0	28.6	2.94	Twist	-42.3	122
13	96	144	2652	38.5	29.1	2.96	Staghead	-40.6	107
14	96	148	2791	39.3	29.2	2.92	Twist	-41.4	99
15	96	134	2709	38.9	30.0	3.42	Staghead	-39.5	108
16	96	145	2954	38.6	29.9	3.33		-39.4	104
17	96	146	2891	39.2	29.7	3.35	Staghead	-39.1	106
18	98	153	2676	38.1	29.4	3.56		-40.7	107
19	97	145	3023	39.7	28.4	3.33		-40.7	108
20	97	146	2478	38.1	29.3	3.23	Twist	-38.9	119
21	97	146	2799	39.4	29.1	3.57	Twist	-37.8	96
AC Vulcan	95	140	2965	41.7	29.9	2.79		-39.1	111
Forge	96	149	2764	39.8	30.1	2.65		-34.5	102
J93-0877	101	142	2823	40.4	26.8	4.40	Twist	-34.7	78

a. Average of three locations. B. Allyl Glucosinolate (µmoles per gram of seed)

Table 2 Selected ZEM lines, cultivars and check in 2000 Co-operative Mustard Trial. Average of 6 locations.

Entry	Yield (% Cutlass)	Oil (% Dry)	Protein (% Dry)	Seed Wt (gm/1000)	Allyl G. (µmole/g)	Colour (WIE)	Chlorophyll (mg/kg)	Maturity (days)	WRR[a] 2a	2v
J97-102 (ZEM)	100	38.0	29.3	2.87	100	-33.5	2.7	98	36	100
J98-326 (ZEM)	97	38.4	28.8	3.84	92	-37.4	10.2	98	60	100
AC Vulcan	101	41.4	30.0	2.93	110	-37.7	2.2	96	10	100
Forge	94	39.4	30.1	2.62	103	-33.6	1.9	96	100	100
Lethbridge 22A	95	41.0	29.8	3.22	78	-36.2	1.7	95	97	100
Cutlass (Check)	100	41.9	29.6	2.93	98	-36.8	1.4	95	14	100

a. Disease reaction of Albugo candida (white rust) on entries in the co-operative mustard trial, incidence of race 2a and race 2v.

Goal 1 is on schedule. We expect that a ZEM cultivar from this project will be registered by 2005. Yield of the recent selections is improved over the original J93-0877 parent line. Seed size is larger. Oil content is generally lower than the parent lines. The stem twisting associated with the original ZEM parent can be selected against in the progeny and it is not linked to the zero erucic acid trial. And the allyl glucosinolate content (hot taste factor in oriental mustard) of the ZEM selections has been increased significantly above the original ZEM parent. Depending on the results from the 2001 multilocation replicated yield; another improved ZEM entry may be made to the 2002 co-operative mustard trial.

Goal 2 - Development of high oleic / low linolenic acid ZEM

White rust resistant oriental mustard development

Of the white rust race 2v resistant oriental mustard lines tested in the 1998 field nursery ten lines were selected on the basis of good agronomic performance, low oil content and high glucosinolate content. These lines were entered into a three location replicated yield trial in 1999. These lines had a yield similar to AC Vulcan and Forge. Their oil content was lower than AC Vulcan, similar to Forge (an oriental mustard variety valued for its low oil content). Their seed weight was generally larger than either AC Vulcan or Forge. And their seed colour was within the normal range of oriental mustard. The allyl glucosinolate content of certain lines also compared favourably with AC Vulcan and Forge. Codes 12 & 13 were entered into the 2000 co-operative mustard trial as J98-12 and J98-48, respectively. Table 3 contains a summary of the performance of these lines in that trial. They outperformed other entries in terms of glucosinolate and protein content. J98-12 also had an excellent oil content, but it did not demonstrate the expected resistance to race 2v in this trial. J98-48 was found to be fully resistant to both races of white rust, but its yield and chlorophyll were unacceptable. J98-12 remains in the co-op trial for the year 2001. Following evaluation of the 2001 co-operative mustard trial and replicated yield trial, selected white rust resistant lines will be crossed with selected a ZEM line (from objective 1) to make white rust resistant (race 2v), zero erucic acid oriental mustard.

Table 3 White rust resistant lines, cultivars and check in 2000 Co-operative Mustard Trial. Average of 6 locations.

Entry	Yield (% Cutlass)	Oil (% Dry)	Protein (% Dry)	Seed Wt (gm/1000)	Allyl G. (µmole/g)	Colour (WIE)	Chlorophyll (mg/kg)	Maturity (days)	WRR[a] 2a	2v
J98-12 (1999 Code 12)	96	39.0	31.0	3.05	11.8	-33.0	1.8	95	6	100
J98-48 (1999 Code 13)	88	40.1	30.9	3.30	11.8	-32.4	5.3	98	0	1
AC Vulcan	101	41.4	30.0	2.93	110	-37.7	2.2	96	10	100
Forge	94	39.4	30.1	2.62	103	-33.6	1.9	96	100	100
Lethbridge 22A	95	41.0	29.8	3.22	78	-36.2	1.7	95	97	100
Cutlass (Check)	100	41.9	29.6	2.93	98	-36.8	1.4	95	14	100

a. Disease reaction of Albugo candida (white rust) on entries in the co-operative mustard trial, incidence of race 2a and race 2v.

High oleic/low linolenic acid ZEM development

The first indications of the cross of ZEM with later generations (BC_4F_4) of high oleic/low linolenic acid, *B. juncea* were more positive than the first attempt. This was probably because of two reasons. Firstly, because a ZEM line was used to cross to the high oleic/low linolenic acid parent, every F_2 half-seed could be evaluated for its oleic acid and linolenic acid content. And secondly, the later generations of the high oleic/low linolenic *B. juncea* were more fertile than the earlier generations, which means more seed was available for half-seed selection, fewer selected plants died or were sterile. Because of the better fertility we were able enter 76 lines of the F_3 generation into a 1999 field nursery and 54 F_4 lines into a 2000 field nursery. The range of fatty composition in the 2000 nursery was not significantly different than that found in the 1999 nursery. However, in general the performance of entries 2000 nursery was better than the previous nursery in 1999, but still poor compared to the checks. Some lines met the criteria of >55% oleic acid. Table 4 lists the fatty composition of harvested seed of selected lines of this nursery compared the low linolenic acid *B. napus* check and ZEM checks that were included in the test. These selected lines demonstrated a significant improvement in fatty acid profile over the ZEM checks. The linolenic acid content of these lines also somewhat improved compared the ZEM checks, but was not close to the level found in the low linolenic acid *B. napus* parent (S86-69). From the glucosinolate data presented it is obvious that further selection for high allyl glucosinolate content or more backcrosses to oriental mustard will be necessary to improve the glucosinolate content to that of oriental mustard.

Table 4 Fatty acid and glucosinolate content of selected lines of high oleic acid, low linolenic acid *B. juncea* of the 2000 nursery

Entry	Plant#	C18:1	C18:2	C18:3	C22:1	Sat'd	Allyl	TotG	% Allyl
7638	T099-5334-23	57.1	19.0	12.6	0.1	7.7	53.28	106.93	49.8
7676	S.P.#30-08	55.1	22.5	12.6	0.0	7.0	45.92	88.47	51.9
7674	S.P.#30-06	52.5	24.2	12.5	0.6	7.1	52.43	92.36	56.8
7639	T099-5338-12	50.6	32.4	7.1	0.0	7.6	41.01	57.77	71.0
7657	T099-5354-20	49.7	28.3	12.4	0.0	6.8	51.29	93.30	55.0
7649	T099-5354-8	49.2	28.6	12.3	0.0	6.9	25.66	47.15	54.4
7656	T099-5354-18	48.8	27.9	12.8	0.0	7.5	48.24	96.06	50.2
7650	T099-5354-9	48.7	28.9	13.2	0.0	6.5	60.84	105.90	57.5
7655	T099-5354-16	48.0	29.2	12.7	0.0	7.2	36.15	74.06	48.8
	ZEM Checks (Avg)	39.1	33.9	17.8	0.1	6.3	108.9	114.3	95.3
7671	S86-69	63.3	25.8	1.7	0.0	6.9	3.15	14.81	21.3
7670	Leth22A	15.9	24.5	13.5	24.7	5.7	74.24	77.08	96.3
7668	Forge	18.7	24.7	12.3	24.4	5.6	98.65	100.89	97.8
7669	AC Vulcan	17.1	21.4	13.9	27.1	5.1	113.52	116.25	97.6

C18:1 = oleic acid (% of total fatty acid); C18:2 = linoleic acid; C18:3 = linolenic acid; C22:1 = erucic acid; Sat'd = Total saturated fat content; Allyl = allyl glucosinolate (µmoles/gm seed); TotG = Total Glucosinolate (µmoles/gm seed); % Allyl = percent of total glucosinolate that is allyl glucosinolate

Low linolenic marker selected BC_4F_2 seed was planted in a nursery in 2000 together with checks. Individual plants in this nursery were bag selfed, tissue sampled for DNA marker analysis and individually harvested. The fatty acid composition of the seed oil of these plants was determined and compared with the presence or absence of the low linolenic acid *B. napus*. The presence of two *B. napus* low linolenic acid markers was correlated with decreased synthesis of linolenic acid from linoleic acid. Elevated levels of oleic acid content were not observed in this population, however. Therefore, a cross with the above high oleic acid ZEM lines or similar lines will be necessary. This will be initiated once the low linolenic content of the marker positive lines is by BC_4F_3 progeny evaluation.

D. Indications for further development

The project is on schedule. After the completion date of this project the selected ZEM line(s) will continue to enter the Co-operative Mustard Trials, so that they can released by the year 2006. In addition, the seed of any potential ZEM cultivar will need to be evaluated by mustard processors to determine it is an acceptable seed source for the manufacture of mustard condiment. Lines of high oleic, low linolenic acid *B. juncea* have been developed which have a significantly improved fatty acid composition compared to the original ZEM lines. However, after the

completion date of the project, further backcrossing to elite oriental mustard and pedigree selection is required on high oleic, low linolenic ZEM. As it lacks in terms of seed quality issues and agronomic performance.

E. Information on performance indicators

The project is on schedule. ZEM cultivar(s) can be expected to enter the marketplace by 2006. High oleic, low linlenic acid ZEM development is showing promise with some lines meeting the minimum requirements. See discussion under headings; "Project activities, achievements, papers written", "Results and conclusions" and "Deliverables for evaluation" for details.

F. Increased market share/sales/production/profits/exports

Can not be measured until a variety of zero erucic oriental mustard is registered and can be marketed. However, if the Japanese mustard processors find the new cultivars of ZEM acceptable for mustard condiment production, they may switch from purchasing normal erucic acid oriental mustard cultivars such as AC Vulcan or Forge to these ZEM cultivars. Countries that use oriental mustard use as an oilseed, such as countries of the Indian subcontinent, may find that ZEM has enhanced to them in the fact that the oil is zero erucic acid like canola oil. As a result Canadian exports to such countries may increase.

G. Increased membership/employment/patents

No application for a patent on ZEM is anticipated at this point.

H. Identification and dissemination of new technological opportunities

When a cultivar of zero erucic acid oriental mustard is registered the availability of the variety will be advertised. At that point interested parties will be able bid for marketing and distribution rights.

I. Increased knowledge of the human resource base

Not applicable

J. Diversification and impact on production/producers/marketplace

Can not be measured until a variety of zero erucic oriental mustard is registered and can be marketed. But zero erucic oriental mustard will allow Canadian farmers to produce another type of oriental mustard. This mustard can be marketed to mustard processors (such as the Japanese mustard processors) which extract a portion of the oil from the mustard seed during processing as having the advantage of increasing of the value of this by-product of their manufacturing process. This is because zero erucic acid mustard oil can be considered to be edible oil, whereas oils, such as the oil from standard varieties of oriental, containing high levels of erucic acid are not. Oil from standard varieties of oriental mustard is not considered to be edible. However, mustard processors who do not remove a portion of the mustard oil during mustard manufacture will not see an advantage in using zero erucic acid mustard seed.

In countries where oriental mustard is used as an oilseed (such as Bangladesh) rather than as a condiment, zero erucic oriental mustard would have the market advantage of producing oil free from a compound (erucic acid) considered unhealthy for humans to consume.

The eventual development high oleic, low linolenic acid, zero erucic acid oriental mustard varieties will allow the oil from this seed to compete in the edible oil market as an oil with properties similar to canola oil.

Personnel

The following personnel

Personnel

G. Serblowski

Deliverables for evaluation

Notes on statements on deliverables in the contract document

"Development of 0 erucic oriental mustard that will enhance marketability of Canadian grown oriental mustard by allowing it to be used for edible oil production in addition to condiment mustard use."

Can not be measured until a variety of zero erucic acid, oriental mustard is registered and can be marketed. But zero erucic acid, oriental mustard will allow Canadian farmers to produce another type of oriental mustard. This mustard can be marketed to mustard processors (such as the Japanese mustard processors) which extract a portion of the oil from the mustard seed during processing as having the advantage of increasing of the value of this by-product of their manufacturing process. This is because zero erucic acid, mustard oil can be considered to be edible oil, while oil from standard varieties of oriental mustard is not considered to be edible, due to the presence of large amounts of erucic acid. However, mustard processors who do not remove a portion of the mustard oil during mustard manufacture will not see an advantage in using zero erucic acid, mustard seed.

In countries where oriental mustard is used, as an oilseed (such as Bangladesh) rather than as a condiment, zero erucic oriental mustard would have the market advantage of producing oil free from a compound (erucic acid) considered unhealthy for humans to consume.

"Development of adapted, high yielding, canola quality *B. juncea* oilseed to add value to oilseed mustard production."

Is sentence does not belong in this document because the focus of this project is on improvements to oriental mustard, not canola quality *B. juncea*. Canola quality *B. juncea* is by definition low in glucosinolate content (less than 18 μmoles of glucosinolate per gram of seed, less than 1 μmole of allyl glucosinolate per gram of seed). Oriental mustard should have an allyl glucosinolate of greater than 100 μmoles per gram of seed, as it is the breakdown of allyl glucosinolate to allyl isothiocyanate in the preparation of mustard that gives mustard its hot flavour. Therefore canola quality *B. juncea* can not be considered oriental mustard.

"Availability of a drought tolerant oilseed/condiment mustard crop will diversify agricultural production of the prairies and generate additional income for farmers"

This can be tested once a variety of zero erucic acid oriental mustard is released into the marketplace. This has not happened and is not expected to happen until after the completion of this project.

"The breeding goal is 5-10% increase in quality (yield, oil, etc.), 5-10% decrease in undesirable qualities and decrease of erucic to 0 for the new germplasm relative to check in the 1997 spring grain variety trial. A cultivar release is expected in 2006. Processor testimonials are an asset. The biotech goal is to develop said techniques to an efficiency level (samples/day), equal to the state of the art for canola in 1997."

The breeding goal of 5-10% increase in yield for zero erucic acid, mustard is well within reach. However, an increase of 5-10% in oil content would not be considered to be an improvement, unless it is being grown specifically for the Bangladesh market. Personal communications from Japanese mustard processors have indicated that while they are interested in ZEM they are not interested in seeing an increase the oil content of this mustard. That is because the value of even edible oil does not equal the value of the dry mustard they produce. So an increase in oil content represents an overall decrease in the value of product they can obtain from the mustard seed. Rather it is our goal to keep the oil content of ZEM as low as possible, as this trait is important to mustard processors. The phrase "the 1997 spring grain variety trial" should read "the 1997 Co-operative Mustard Trial".

Notes on yearly goals

"1997 - basic germplasm development through cross breeding and selection for 0 erucic acid oriental mustard with: high oleic/low linolenic, improved plant type, high allyl glucosinolate and resistance to white rust 2v."

Progress was made on these fronts. However, it should be pointed out that it was not our intention that all these desirable traits would be in one line/cultivar by the completion date of this project. As such a task can not be completed within 4 years. Rather it is our intention that by the end of this project there will be at least one variety of zero erucic acid, oriental mustard developed. It will be competitive with the standard oriental mustard varieties in terms of yield (agronomic performance) and in terms of seed quality (low oil content, low chlorophyll content, high protein content, high allyl glucosinolate content, possibly larger seed size). It is expected that by the completion date of this project that these lines will be ready to be entered into the Co-operative Mustard trials for possible registration and release by the year 2006. These lines will not be particularly higher in oleic acid and lower in linolenic acid than our ZEM parent line.
Secondly, it is our intention to introgress the high oleic / low linolenic acid trait from *Brassica napus* into ZEM through a interspecific cross of canola quality *B. juncea* with *B. napus* followed by backcrosses to oriental mustard. We expect that by the completion date of this project we will have field tested such lines and found lines of zero erucic mustard that have an oleic acid content of greater than 55% of total fatty acids in its seed oil. These lines will also have an linoleic acid content of less than 25%, and a linolenic acid content of less than 10%. Reaching these levels would mean that the oil of this mustard seed could be considered to equivalent to canola oil in quality. These lines will not be ready for entry into the co-operative mustard trials, but rather we will be satisfied they just survive under field conditions and produce seed. Even this would represent an improvement in plant type, since as yet we've have not be able to demonstrate survival under field conditions with high oleic/low linolenic, low glucosinolate *B. juncea*. These lines will have to undergo cytological examination to prove they are indeed *B. juncea*.
Finally, it is our intention to introgress resistance to white rust race 2V from *B. napus* into oriental mustard through the interspecific cross of canola quality *B. juncea* with *B. napus* followed by backcrosses to oriental mustard. By the end of product we expect to have lines of zero erucic mustard that are resistant to white rust race 2V. Again these lines will have to undergo cytological examination to prove that they are indeed *B. juncea*.

1998- continue basic germplasm development for 0 erucic acid oriental mustard through crossbreeding and selection

Good progress has been on all three fronts in 1998. A field nursery of selected ZEM lines was grown, from which selections will be made for further testing and advancement. There will also be another nursery in 1999 to provide a second cycle of selection.

Crosses of high oleic/low linolenic acid *B. juncea* were made to ZEM and half-seed selections for the high oleic/linolenic acid trait made. F_3 seed of selected plants will be planted in a nursery for evaluation under field conditions. Selected plants will also be further backcrossed to ZEM to improve agronomic performance. The high oleic/low linolenic acid trait will have to be reselected in 1999 within the backcross generation. Cytological evaluation of selected lines is planned for the last year of the project.

White rust (race 2v) resistant oriental mustard was field tested for the first time in 1998. The overall performance of the entries in the nursery was good, which bodes well for the other oriental mustard material we developed from interspecific crosses with *B. napus*, including high oleic/low linolenic acid ZEM. Cytological evaluation of selected lines is planned for the last year of the project.

1999

Good progress has been on all three fronts in 1999. Both a field nursery and a multilocation replicated yield trial were grown, from which selections will be made for further testing and advancement. A ZEM line with yield similar to AC Vulcan may also be entered into the 2000 Co-operative Mustard Trial, one year ahead of schedule. There can also be another mulitlocation yield trial of selected lines and another nursery.

A replicated white rust (race 2V) resistant multilocation yield trial was also grown. From which a selection or selections can be entered into the 2000 Co-operative Mustard Trial. This development may lead to a new variety of standard oriental mustard with improved white rust resistance, in addition to a ZEM line with white rust (race 2V) resistance. These lines will be used parents for crossing with ZEM line(s) mentioned above and high oleic acid, low linolenic acid lines mentioned below.

Development of high oleic acid, low linolenic acid *B. juncea* also continued on course. A field nursery was grown of 76 lines of which several lines had a fatty acid profile that demonstrated a significant improvement over ZEM. One line exceeded our final goal for this project which was to had a line or lines of ZEM with a fatty acid profile containing greater than 55% oleic acid, less than 30% linoleic acid and less than 12% linolenic acid. Another cycle of selection for high oleic acid, low linolenic acid content and a normal glucosinolate was also carried out on selected F_3 lines. As expected, we saw further segregation within these lines for fatty acid composition and for glucosinolate content. Another nursery of new selections from this material will also be grown in 2000 to evaluate our progress. Further selection and/or backcrossing to ZEM with reselection for high oleic acid, low linolenic acid profiles will continue with this material. Marker selected plants of low linolenic *B. juncea* backcrossed to ZEM will also be in a field nursery in 2000. Individual plants will be harvested and evaluated for their oleic acid and linolenic acid content. From that evaluation a better low linolenic acid parent may be found which will be used in further crosses with our high oleic acid, low linolenic acid lines.

2000

Progress continued on all fronts in 2000. One ZEM line (J98-326) was selected from the results of the 1999 replicated yield trial and entered into the 2000 co-operative mustard trial. It was dropped, however, from the entries for the 2001 trial possible because of high chlorophyll content. Another ZEM line (J97-102) continues in the 2001 co-operative trial for more evaluation and possible registration. Large seeded ZEM and high oleic acid ZEM nurseries were grown in 2000. And a replicated yield trial of large seeded ZEM lines was grown. One entry from this trial has been entered into the 2001 co-operative trial. From the 1999 replicated yield trial of white resistant oriental mustard two lines were entered into the 2000 co-operative trial. These two entries continue in the 2001 co-operative trial. In addition high oleic acid oriental mustard continued to be advanced to BC_5F_5 and BC_6F_1 generations. Marker assisted selection was carried out through 3 successive backcrosses to ZEM and the BC_4F_2 generation was grown in the field, harvested and the correlation of the presence of *B. napus* low linolenic markers with actual linolenic acid content determined.